The effectiveness and safety of vaccines against human anthrax: a systematic review

Vittorio Demicheli*, Daniela Rivetti*, Johnathan J. Deeks†, Tom Jefferson‡§ and Mark Pratt‡

We report on the results of a systematic review of existing controlled clinical trials undertaken to assess the effectiveness and safety of vaccines against human anthrax in relation to disease incidence and side-effects. Two articles retrieved by electronic and hand search fulfilling some of the inclusion criteria underwent a quality assessment by a group of reviewers. Data synthesized from the two trials showed that estimates of overall effectiveness and safety favour treatment (overall odds ratio 0.16; 95% confidence interval 0.07-0.34). The route of inoculation appears to make little difference to the effectiveness of the vaccines; however, one study shows that the incidence and severity of side-effects are significantly higher with the killed vaccine than with the alum-based placebo (overall odds ratio 0.16; 95% confidence interval 2.38-27.17).
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Keywords: anthrax vaccines: systematic review: clinical trials

Anthrax is an acute bacterial skin disease caused by *Bacillus anthracis*, which may rarely involve the respiratory and gastro-intestinal tract. Cutaneous disease mainly ends with the expression of a painful eschar, but, if left untreated, cutaneous anthrax has a mortality rate of up to 20%, caused by septicaemia and meningitis. The mortality rate of the respiratory form approaches 100%[1].

Anthrax is a primary disease of herbivores, mainly animals ingesting or inhaling *B. anthracis* spores while feeding. When terminally ill, such animals shed bacilli with blood and body fluids and, once exposed to the outside environment, these produce hardy spores. Humans mainly contract the disease when exposed to hides of animals containing spores, which can survive for years in skin and bone. Anthrax is therefore mainly a disease of agricultural workers and an occupational hazard of tanners and veterinarians. Anthrax is found throughout the world, but concentrated in Europe, Africa and Asia[2].

Interventions against anthrax range from prevention, through education of operators and dust control, to treatment with penicillin, disinfection and isolation of suspected cases. Human vaccines against *B. anthracis* were developed in the 1950s and 1960s. At present, three vaccines are commercially available: the Georgian/ Russian, the UK and the USA vaccines.

* Agenzia Regionale per la Protezione Ambientale del Piemonte, Area di Epidemiologia Ambientale, Via Santa Caterina da Siena, 30-15100, Allesandria, Italy. †Centre for Statistics in Medicine, Institute of Health Sciences, Old Road, Headington, Oxford, OX3 7LF, UK. ‡Army Medical Directorate 5, Ministry of Defence Building 21a, Keogh Barracks, Ash Vale, Hants, GU12 5RR, UK. §To whom all correspondence should be addressed. Tel. ++44 1252 340 335; fax: +44 1252 340 368; e-mail: zorria@epinet.co.uk.
(Received 16 November 1997: accepted 18 December 1997)

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Modern understanding of the pathogenic mechanism of anthrax revolves around the three components of the bacterial toxin: protective antigen (PA), lethal factor (LF), and odedema factor (EF). PA is undoubtedly the most important immunogen, as high levels of anti-PA are thought to confer protection. However, PA on its own is thought not to be sufficient to stimulate immunity and other non-specific antigens are necessary[3].

The Georgian/Russian vaccine is produced by the Tblisi Research Institute of Vaccines and Serums and consists of live spores from a Stern strain of *B. anthracis* administered in the shoulder by scarification. Its efficacy is unknown but it is reputed to have a high number of side-effects and contra-indications to its use. Apparently, other sites in the former USSR are also involved in the production of similar vaccines (Dr. Tim Brooks, personal communication).

The UK and USA vaccines consist of alum-precipitated cell-free filtrates of bacilli (so-called supernatant particles). The US vaccine, manufactured by the Michigan Department of Public Health, is adsorbed onto aluminum hydroxide. Both vaccines are rapidly grown to minimize the content of LF and EF and to maximize the PA content. Although man is thought to be relatively resistant to anthrax, the effectiveness of both vaccines is unknown but thought to be high, while side-effects are thought to have a low incidence. In the UK, vaccination is recommended only for workers at risk of exposure to anthrax (personnel of tanneries processing wool or hides and bone). The vaccine is administered with four injections of 0.5 ml with intervals of three weeks between the first three injections and 6 months between the third and fourth. A booster dose should be given yearly[3].

Although the first recorded immunization against anthrax in animals dates back to 1880[3], until recently there has been little research into anthrax vaccines other than that carried out for anti-bacteriological warfare purposes by the military. In April-May 1979 there was an outbreak of anthrax in the area immediately downwind of a military microbiological facility at Sverdlovsk in Russia. Ninety-six cases of anthrax were reported: of these 64 died[5]. This outbreak is likely to have been a consequence of the accidental release into the air of a cloud of spores of bacilli from a military establishment connected with the use of *B. anthracis* for biological warfare purposes. In 1990-1991, British troops deployed during the Gulf War were immunized with the UK vaccine, as a precaution against possible use of *B. anthracis* as an airborne weapon by the Iraqis.

In the search for new vaccines, manufacturers have aimed for better quality purified PA vaccines with better adjuvants: vaccines made through recombinant gene technology (*B. Subtilis*, baculovirus or Vaccinia virus expressing the PA gene); and mutant vaccines with altered PA, EF, and LF sequences to render them avirulent but immunogenic.

Despite these developments, several issues are still unresolved concerning the overall comparative effectiveness and safety of old and new vaccines.

This paper aims to assess the effectiveness and safety of vaccines against human anthrax by means of a systematic review of existing literature according to the guidelines of the Cochrane Collaboration[6]. As the most reliable information on any type of health care intervention is provided by the result of randomized clinical trials (RCTs), we assessed the ability of anthrax vaccines

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to induce side-effects and protect from disease compared with no intervention, placebo, or vaccines against other disease (control vaccines).

The following hypotheses were tested:

(1) There is no difference in the number of cases of anthrax occurring in a placebo arm or in other control arm compared to a vaccinated arm of a trial.
(2) There is no difference in the number and severity of side-effects (both systemic and localized) following anthrax vaccination, occurring in a placebo or other control arm compared to a vaccinated arm of a trial.

MATERIALS AND METHODS

The following criteria for considering studies for the review applied:

- prospective randomized or quasi-randomized studies comparing anthrax vaccines in humans with placebo, control vaccines or no intervention;
- studies in which participants were well adults or children, irrespective of immune status or special risk category (tannery personnel, agriculture workers, persons exposed to anthrax either during an epidemic or accidentally);
- studies in which both live and killed vaccines or fractions thereof were administered by any route.

As outcome measures we considered numbers of cases of anthrax avoided by vaccination (specifically incidence of anthrax in the intervention and non-intervention groups) and number and seriousness of side-effects (classified as local and systemic) in different arms of the trial(s). We also considered including antibody titre rise as a surrogate outcome, but as none of the included studies reported serological effects of the vaccines (see below) this outcome was not included.

A case was defined clinically and the denominator was person-months of potential exposure. In trials where exposure was not recorded, the number of cases divided by the number vaccinated/not vaccinated in the defined geographical study area were compared.

Systemic side-effects included cases of malaise, nausea, fever, arthralgias, rash, headache, and more generalized and serious signs. Local side-effects comprised only prominent local reactions, i.e., those associated with the development of local oedema at the site of inoculation.

We carried out an electronic search of MEDLINE using a Cochrane standard search strategy of 34 MESH terms or combined sets from 1966 in any language described elsewhere[7].

We read the bibliography of retrieved articles in order to identify further trials. We carried out a search of EMBASE and handsearched the journal *Vaccine* from its first issue to the end of 1995[8]. In order to locate unpublished trials we wrote to the manufacturers of vaccines and researchers active in the field.

All possible trials identified were read and checked by six independent assessors. Standard Cochrane Infectious Diseases Group method[6] for assessment of trial quality was used to assess the following four dimensions:

(1) generation of allocation schedule.
(2) measure(s) taken to conceal treatment allocation.
(3) exclusion of allocated participants from the analysis of the trial.
(4) measures taken to implement and protect double blind.

(3)

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For criteria 2, 3, and 4 there is empirical evidence that low quality in their implementation is associated with exaggerated trial results and it is reasonable to infer a quality link between all four items[9]. Assessment took place using a set of parameters agreed prior to the reviewers having access to the studies.

Disease rates were calculated as the ratio of the number of cases to the number of participants included in each group, which ignores any between-trial variation in effectiveness related to different lengths of follow-up. As cases are very rare, the relative rate is very similar to the odds ratio, which was used to combine the study results. All figures quoted were calculated using the Mantel-Haenszel odds ratio, which gives more reliable results than the Peto method when placebo and experimental arms are not strictly balanced.

RESULTS

The search strategy retrieved three articles[10,12]. Only two fulfilled the criteria for considering trials for the review[10,11]. The characteristics of these studies are reported in *Table 1*. Neither study reported antibody titre rise as an outcome.

Quality of study reporting was not good: both studies had unclear allocation concealment procedures and reported taking inadequate measures to protect double blinding. However, the Brachman study[11] had adequate measures to deal with withdrawals from the study and the Burgasov study[10] reported an adequate system of random cluster sampling and stratification by baseline risk in the 228 localities in which the trial had taken place.

The quality of the Brachman study was relatively good: although randomization is not mentioned in the text, placebo was administered to the control arm in a single blind fashion. The Burgasov study had a good description of the randomization process, but no mention was made of blinding or how withdrawals from the trial were taken into account. Quality assessment judgements showed a high level of inter-reviewer agreement with non-significant differences in score variance.

Although the Burgasov (1976) trial[10] was cluster randomized (see *Table 1*), it was not possible to make an appropriate adjustment in this analysis due to lack of information in the original trial. The impact of ignoring cluster randomization depends on the independence of the anthrax cases within the study. If some of the cases did occur with the same villages, the precision of the treatment effect is overestimated, but given the very low disease rates it seems unlikely that this would substantially change the conclusions.

Figure 1 shows the effectiveness of anthrax vaccines in avoiding cases of illness. *Figure 2* shows the safety of killed vaccine compared with placebo.

Estimates of overall effectiveness and safety favour treatment, the odds ratio (OR) for avoided cases of anthrax being 0.16 (95% confidence interval (CI); 0.07-0.34). As the calculation of the relative risk (RR) and the OR yield identical results, anthrax vaccines appear 84% effective in avoiding cases. A chi-squared test of the two studies included in the meta-analysis showed no significant difference between the results of the two trials (*Figure 1*. chi-square 0.18 with 1 degree of freedom or df = 1).

The potential impact of the vaccination varies between setting. In the high incidence setting of the mills in the Brachman study (1.2 cases per 100 per year), vaccination of 99 individuals will prevent one case of anthrax per year. In the lower incidence setting of the Kazakh trial

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Table 1. Characteristics of included studies

Study	Brachman	Burgasov
Methods	Single-blinded placebo-controlled study	Random cluster intervention study of anthrax vaccine versus placebo in three arms
Participants	1,249 workers in four tanneries in the North-East of the USA in the 1950s. 379 workers were vaccinated, 414 were assigned to placebo. Additionally, 116 workers did not complete the vaccination cycle and 340 refused to take part in the study	157,259 persons in the Kazakh Soviet Socialist Republic spread in 228 sites in which anthrax was endemic. 52,763 people were assigned to vaccination by needleless injection, 54,522 were assigned to vaccination by scarification and 49,974 were controls (no intervention)
Interventions	Protective antigen (R1-NP strain) vaccine made from sterile culture filtrate produced by the US Army. 0.5 ml was administered subcutaneously in three doses at weeks 0, 2, and 4. The primary cycle was followed by three booster doses (0.5 ml) at 6- monthly intervals. Placebo contained a solution of 0.1% alum	STI-1 strain probably attenuated vaccine administered either by needleless gun or by scarification (scraping of the skin) compared to no vaccination. No vaccination schedule is given. Participants were followed-up for 2 years
Outcomes	Cases of anthrax, incidence and severity of side-effects (divided into local and systemic). A "clinical" case definition was used. Although the study was carried out between 1955 and 1959, the average length of exposure was 16 months, given the rolling nature of the immunization programme. Overall the vaccine was found to be 92.5% effective in preventing cases of anthrax. Separate effectiveness estimates for cutaneous and inhalation forms are not given. Systemic reactions occurred in 0.5% of cases, while local reactions occurred in 5.5% of cases	Anthrax cases. Case definition was "bacteriological". STI vaccine afforded 75.0% protection when vaccinated by scarification and 84.2% when vaccinated by the needleless gun method
Notes	The incidence of anthrax was 1,200 per 100,000 per year in the four tanneries	The annual incidence of anthrax in the former USSR Republic of Kazakh was 15.4 cases per 100,000 per year

(15.4 cases per 100,000 per year) vaccination of 7730 subjects is needed to prevent one additional case of anthrax.

Brachman showed that the killed vaccine was found to be 92.5% effective in preventing cases of anthrax. Separate effectiveness estimates for cutaneous and inhalation forms are not given but the small numbers of inhalation anthrax found in their study led them to be unable to conclude protective benefit. Local prominent reactions (typically; erythema, induration and oedema at the site of inoculation) occurred in 5.5% of cases and lasted 24-48 h while systemic reactions occurred in 0.5% of cases (with local oedema and malaise of 24 h duration). Two of three vaccinees who had natural immunity to anthrax experienced severe local reactions lasting up to 48 h.

The incidence and severity of side-effect was different between the killed vaccine and control alum-based placebo (OR 10.45; 95%, CI 3.45-31.69). Burgasov reports that the STI vaccine afforded 75% protection when administered by scarification and 84.2% when administered by the needleless gun method. This difference in outcome was hypothesized to occur because of non-standard application of scarification but was not statistically significant (OR 1.62; 95% CI 0.39-6.67).

DISCUSSION

The results of our review show that there are several limits to the knowledge of the effects of anthrax vaccines. There appear to be few comparative studies available. Available studies assess the older generation of vaccines and show several **(5)**

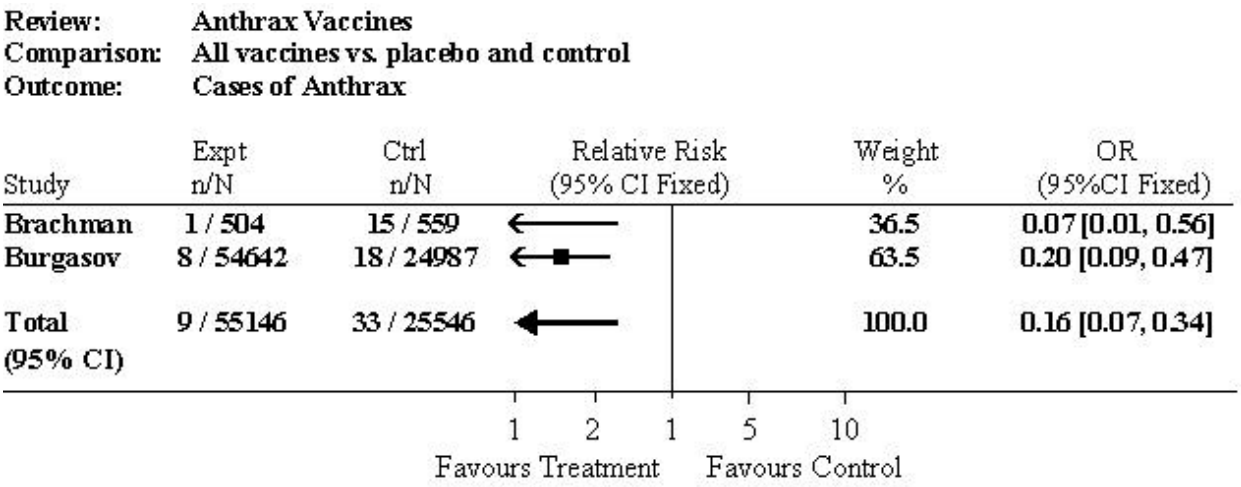

Figure 1. Comparison of any vaccine with placebo/control using cases of anthrax as outcome measures. The figure contains the proportion of cases among vaccinated (Experimental or Expt) and unvaccinated (Control or Ctrl) subjects observed in each study and the pooled result of the meta-analysis. The relative effect of the vaccine is expressed by means of the value of the odds ratio (OR) and its 95% confidence intervals (CI). The weight of each study included in the meta-analysis depends on the size and quality of the study.

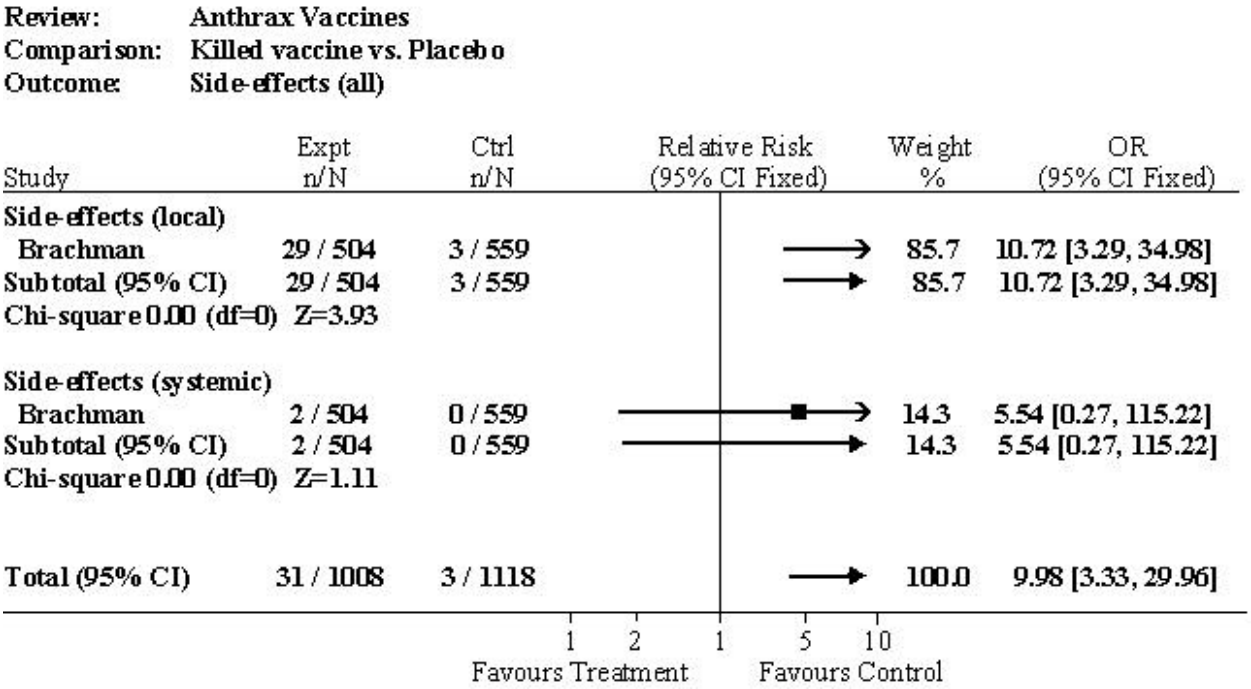

Figure 2. Comparison of killed anthrax vaccine with placebo/control using side-effects as outcome measure. The figure contains the proportion of side-effects among vaccinated (Experimental or Expt) and unvaccinated (Control or Ctrl) subjects observed in each study and the pooled result of meta-analysis. The relative safety of the vaccine is expressed by means of the odds ratio (OR) and its 95% confidence intervals (CI). The weight of each study included in the meta-analysis depends on the size and quality of the study.

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methodological weaknesses such as uncertain case definitions, unclear vaccination schedules and weak experimental design. Despite such weaknesses and the fact that the two studies in this review assess two different types of vaccines (killed and attenuated), we believe that the data presented in this review show that overall anthrax vaccines are safe and efficacious. The major share of the weight of our conclusions belongs to the study by Burgasov *et al*. which is a large, relatively well-designed study carried out in a relatively high-incidence (1.5 per 100 per year) republic of the former USSR. In this study, a live attenuated vaccine is used which is different from the current fractionated supernatant vaccines in service in the UK and USA. However, we do not see a case for further experimentation of existing vaccines for this very rare disease. We are, however, disappointed in our inability to identify trials of the newer vaccine formulations, but have no reason to believe that such trials exist. We have commenced personal contacts with Russian officials in an attempt to locate and retrieve any studies unknown to us.

We conclude that killed anthrax vaccine is efficacious and well tolerated and should be administered to persons at high risk of the disease. Although experimentation on humans will be necessary for new vaccines, no further experimentation on the old killed vaccines should be carried out.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. Chester Morris, Patricia Graves, Alaistair MacMillan, Ron Behrens, Paul Garner, Tim Brooks, Franco Bottasso and Gaving Young and Mrs. Carol Hobbs for help received. This study was funded by the Ministry of Defence of the United Kingdom.

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